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ATROPINE-RESISTANT SUBMANDIBULAR RESPONSES TO STIMULATION OF THE PARASYMPATHETIC INNERVATION IN THE ANAESTHETIZED FERRET

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SUMMARY

1. Submandibular salivary and vascular responses to stimulation of the peripheral end of the chorda-lingual nerve at 20 Hz continuously for 60 min were investigated in anaesthetized ferrets, in which the sympathetic innervation to the gland was cut, in the presence and absence of atropine (2·0 mg kg^{-1}).

2. Both the increase in submandibular salivary flow and protein output, which occurred in response to nerve stimulation, were substantially reduced following the administration of atropine, the latency was greatly increased thereby, and both responses were more transient but neither was abolished by atropine. The fall in submandibular vascular resistance was not significantly affected by atropine, either in respect of extent or duration.

3. Chorda-lingual stimulation produced an increase in the output of vasoactive intestinal peptide (VIP), substance P (SP) and calcitonin gene-related peptide (CGRP) in the submandibular venous effluent blood. Each of these responses was maximal within the first 10 min after the onset of stimulation and declined thereafter. The time-scales of both the CGRP and SP responses were similar to those of the atropine-resistant secretory responses, both being quite short-lived, whereas the output of VIP (like the atropine-resistant vascular response) was significantly greater than the basal value throughout the whole of the 60 min period of stimulation.

4. The CGRP response was completely abolished by pre-treatment with atropine, whereas the outputs of both VIP and SP were significantly enhanced thereby. Both the submandibular vascular and secretory responses to chorda-lingual stimulation were almost completely suppressed following the administration of hexamethonium, and there was then no detectable release of peptidergic agonists from the gland.

5. The atropine-resistant submandibular salivary secretory responses were completely abolished by pre-treatment with a tachykinin inhibitor ([D-Arg^1, D-Cl^2 Phe^5, Asn^6, D-$Trp^{7,9}$, Nle^{11}]-SP; 0·75 mg kg^{-1}) without affecting the fall in submandibular vascular resistance.

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6. Following pre-treatment with hexamethonium, I.V. bolus injections of methacholine, SP and CGRP elicited increases in submandibular blood flow and secretion of saliva. VIP caused an increase in blood flow without overt secretion, although it is known to increase secretion of protein and to potentiate the secretory response to SP. Taken together, all these results are consistent with the contention that VIP contributes to the vasodilator response to stimulation of the parasympathetic innervation in this gland and that both SP and CGRP are likely to contribute to the secretory response.

INTRODUCTION

Orthodoxy, deriving from Heidenhain's classic study (Heidenhain, 1872), is that salivary secretory responses to stimulation of the parasympathetic innervation are abolished by atropine, whereas the vasodilator response is not. In the submandibular gland of the cat, this atropine-resistant phenomenon can be accounted for by the release of vasoactive intestinal peptide (VIP) from postganglionic parasympathetic nerve terminals (Bloom & Edwards, 1980; Lundberg, Ånggård, Fahrenkrug, Hökfelt & Mutt, 1980). However, more recently, evidence has been obtained that there is also an atropine-resistant component involved in salivary secretory responses to parasympathetic stimulation in several species including the rat (Thulin, 1976; Ekström, Månsson, Tobin, Garrett & Thulin, 1983), sheep (Reid & Titchen, 1988) and ferret (Ekström, Månsson, Olgart & Tobin, 1988*c*).

In the submandibular gland of the ferret pre-treated with adrenoceptor antagonists, atropine-resistant secretory responses may amount to as much as 30% of those which occur in response to parasympathetic stimulation in the absence of the muscarinic receptor blocking agent (Ekström *et al.* 1988*c*). Such atropine-resistant secretory responses are demanding of high frequency stimulation and can rapidly be fatigued by prolonged parasympathetic stimulation. It is likely that tachykinins, VIP and calcitonin gene-related peptide (CGRP) are involved in the atropine-resistant secretory response to stimulation of the parasympathetic innervation of the submandibular gland in the ferret, in virtue of the following observations. (1) Intravascular infusions of substance P (SP) provoke a copious flow of submandibular saliva in this species (Ekström *et al.* 1988*c*). (2) Intravascular infusions of VIP stimulate secretion of protein from the acinar cells, without eliciting any flow of saliva (Ekström & Tobin, 1989). (3) Combined stimulation with SP and VIP has shown that VIP strongly potentiates the secretory responses to SP. (4) Immunocytochemical techniques have indicated the presence of VIP, SP and CGRP in nerve terminals abutting acini, ducts and blood vessels in this gland (Tobin, Luts, Sundler & Ekström, 1990*b*).

All this evidence suggests that these neuropeptides may be involved as autonomic transmitters in the control of salivary secretory or vascular responses. Accordingly, the present study was undertaken to establish whether these peptides are released within the gland in response to parasympathetic stimulation and the extent to which submandibular responses to such stimulation is affected by pre-treatment with (a) atropine and (b) a tachykinin antagonist. Certain of these results have been published previously in a preliminary form (Tobin, Ekström, Edwards & Bloom, 1990*a*).

METHODS

Animals

The experiments were carried out on twenty-nine adult male ferrets (1·9 ± 0·1 kg body weight; mean ± S.E.M.) from which food, but not water, was withheld for 16–24 h before each experiment. The animals were anaesthetized with sodium pentobarbitone (Sagatal; May & Baker; 45 mg kg^{-1}, I.P.) followed by supplementary doses injected intravenously as required.

Preparatory surgery

The trachea was cannulated and body temperature was maintained at about 38 °C by means of a thermostatically controlled blanket connected to a thermometer inserted into the rectum. The parasympathetic chorda-lingual nerve was exposed and cut as far proximal as possible before the peripheral end was attached to a bipolar platinum electrode. A fine glass cannula was placed in the submandibular duct taking care to ensure that the adjacent chorda tympanic nerve fibres were not damaged. Each of the tributaries of the external jugular vein, except for those draining the submandibular gland, were ligated and the animals were heparinized (750 i.u. kg^{-1}, I.V.) prior to cannulation of the jugular vein with a short length of polyethylene tubing whereby the submandibular venous effluent was diverted through a photoelectric drop-counter. The submandibular effluent blood was returned to the animals, via a catheter placed in a femoral vein, by means of a pump (Gilson, Minipuls 3), at a rate which closely matched output to input, making allowance for periodic collection of blood for analysis; the volume of blood removed for this purpose (both venous and arterial) was carefully monitored, and compensated for by the addition of appropriate volumes of Dextran 40 (Pharmacia, Uppsala, Sweden).

Experimental procedures

The chorda-lingual nerve was subsequently stimulated at 20 Hz (8 V, 5 ms) for 45 or 60 min in four groups of animals under different sets of experimental conditions. These comprised a control group given no pharmacological blocking agent ($n = 10$), a group pre-treated with atropine (2·0 mg kg^{-1}; $n = 10$), a group pre-treated with both atropine and a tachykinin antagonist [D-Arg^1, D-Cl^2 Phe^5, Asn^6, D-$Trp^{7,9}$, Nle^{11}]-SP (0·75 mg kg^{-1}, I.V.; Ekström, Häkansson, Månsson & Tobin, 1988*b*; $n = 4$) and a group pre-treated with the ganglion blocking agent hexamethonium (20 mg kg^{-1}; $n = 5$). In this last group the capacity of the gland to respond to cholinergic and peptidergic agonists directly was tested by giving bolus injections of methacholine, CGRP, SP and VIP I.V. via the femoral vein. Drops of saliva falling from the tip of the submandibular salivary cannula were recorded photoelectrically and collected into pre-weighed tubes in order that the flow could be estimated gravimetrically (assuming the density of saliva to be 1·0 g ml^{-1}). Submandibular blood flow was also estimated gravimetrically. Mean aortic blood pressure and heart rate were monitored continuously, by means of a pressure transducer connected to a cannula inserted into the right femoral artery. Arterial packed cell volume was measured at intervals throughout each experiment. Effects of possible fluctuations in adrenergic activity in the gland were eliminated by removal of the ipsilateral superior cervical ganglion. Drugs and peptides were employed as follows: atropine sulphate, hexamethonium bromide and methacholine chloride (Sigma Chemical Co.), dihydroergotamine methansulphonate (Sandoz AG), propranolol hydrochloride (ICI), SP (Peninsula Laboratories), [D-Arg^1, D-Cl^2 Phe^5, Asn^6, D-$Trp^{7,9}$, Nle^{11}]-SP (kindly donated by Ferring Pharmaceuticals, Malmö, Sweden; Ekström *et al.* 1988*b*) and VIP (kindly supplied by Professor V. Mutt, Karolinska Institutet, Stockholm, Sweden).

Measurements and analyses

Samples of submandibular saliva were collected at intervals and estimated for protein content by the method of Lowry, Rosebrough, Farr & Randall, (1951), using bovine serum albumin as the standard. Samples of submandibular venous effluent and arterial blood were also collected at intervals into ice-chilled, pre-weighed tubes containing aprotinin (Trasylol, Bayer; 2500 K.I.U. (ml $blood^{-1}$) for peptide assays. The tubes were then centrifuged at +4 °C as soon as possible and the plasma sequestered at −20 °C. VIP, SP and CGRP were measured by radioimmunoassays as previously described (Mitchell & Bloom, 1978; Bloom & Long, 1982; Bloom, Edwards & Jones, 1989). Peptide output was estimated from the submandibular arterio-venous difference in concentration and plasma flow, and expressed in fmol min^{-1} (g $gland)^{-1}$. Submandibular vascular

resistance was calculated by dividing the perfusion pressure (taken to be aortic blood pressure during collection of the submandibular blood sample) by submandibular blood flow at that time.

Statistics

Statistical significance was determined by the Wilcoxon signed rank test (paired comparisons in individual animals) or the Mann–Whitney U test (interindividual comparisons) and calculations were invariably made on raw data. P values of 0·05 and less were regarded as statistically significant.

RESULTS

Secretion of submandibular saliva and protein

Stimulation of the peripheral end of the chorda-lingual nerve at 20 Hz continuously provoked a flow of submandibular saliva, together with an increase in protein output both in the presence and absence of atropine (2·0 mg kg^{-1}; Fig. 1). In both groups of animals these responses peaked within the first 5 min, declining steadily thereafter. However, pre-treatment with atropine substantially increased the latency of these responses and reduced their extent (Fig. 1). Thus, secretion was first observed $2{\cdot}8 \pm 0{\cdot}9$ s after the onset of stimulation in the absence of atropine compared with a time-lapse of $35{\cdot}0 \pm 3{\cdot}3$ s in the atropinized group ($P < 0{\cdot}001$). Mean salivary flow during the initial collection period (0–5 min) amounted to 254 ± 32 μl min^{-1} (g gland)$^{-1}$ in the control group compared with a mean value of 90 ± 12 in the group which were given atropine ($P < 0{\cdot}001$).

In the absence of atropine salivary flow declined fairly steadily between 5 and 30 min and maintained a relatively steady level thereafter, amounting to 63 ± 15 μl min^{-1} (g gland)$^{-1}$ at 55–60 min; the total volume of saliva secreted under these conditions was $4{\cdot}5 \pm 0{\cdot}6$ ml (g gland)$^{-1}$. In the atropinized group the comparable total volume was significantly lower ($0{\cdot}5 \pm 0{\cdot}1$ ml (g gland)$^{-1}$; $P < 0{\cdot}001$) and had virtually ceased after 30 min (Fig. 1). The output of protein changed roughly in parallel with the secretion of fluid, in the absence of atropine, resulting in a fairly steady salivary protein concentration. However, after pre-treatment with atropine, there was a gradual increase in protein concentration during continued stimulation, reflecting the fact that the rate of reduction in flow exceeded that in protein output (Fig. 1).

Submandibular blood flow

Atropine had no effect, either on resting submandibular blood flow, which amounted to about 0·3 ml min^{-1} (g gland)$^{-1}$ in both groups of animals, or on the latency or extent of the vascular response to chorda-lingual nerve stimulation (Fig. 2). Initially, submandibular blood flow rose to about 3 ml min^{-1} (g gland)$^{-1}$ (0–10 min) and was maintained at about 2 ml min^{-1} (g gland)$^{-1}$ thereafter. These changes in blood flow reflected closely similar falls in submandibular vascular resistance in the two groups with no significant difference between the mean perfusion pressures, although mean aortic blood pressure was slightly lower in the atropinized animals initially.

Submandibular peptide outputs

In the absence of atropine, stimulation of the peripheral end of the chorda-lingual nerve at 20 Hz for 60 min elicited a prompt, if transient, increase in the outputs of VIP, SP and CGRP from the gland, which was statistically significant, during the

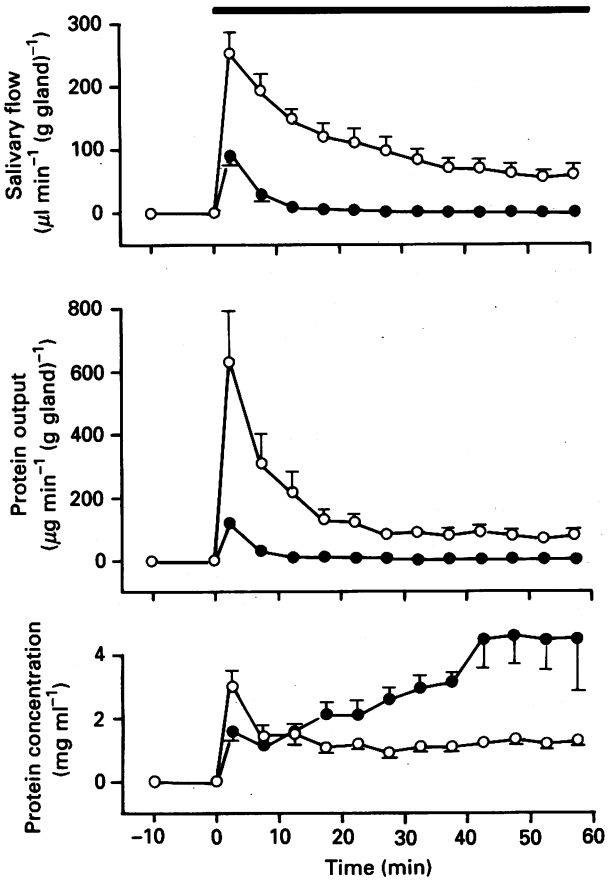

Fig. 1. Comparison of the changes in mean submandibular salivary flow, protein output and protein concentration in response to continuous stimulation of the peripheral end of the chorda-lingual nerve at 20 Hz for 60 min in anaesthetized ferrets. ○, normal control animals ($n = 10$). ●, animals given atropine (2·0 mg kg^{-1}; $n = 10$). Horizontal bar, duration of stimulation. Vertical bars, S.E. of each mean value.

initial 5 min collection period, in each case ($P < 0{\cdot}01$ or better). The outputs of SP and CGRP fell back towards the initial values relatively rapidly, in spite of continued stimulation, and were both reduced to about 10% of the mean peak values after 15 min. The output of VIP also faded but less rapidly, being reduced only to about 65% of the mean peak values at 15 min and to about 10% after 40 min (Fig. 3).

The outputs of both VIP and SP were substantially increased by prior treatment with atropine and this enhancement was statistically significant in each case. Thus, the mean total output of VIP in atropinized ferrets was $7{\cdot}2 \pm 2{\cdot}0$ pmol (g gland)$^{-1}$ compared with a value of $2{\cdot}9 \pm 0{\cdot}7$ pmol (g gland)$^{-1}$ in the absence of atropine ($P < 0{\cdot}05$), over the 60 min period of stimulation. The corresponding values for SP were $2{\cdot}5 \pm 0{\cdot}8$ pmol (g gland)$^{-1}$ (with atropine) and $0{\cdot}9 \pm 0{\cdot}4$ pmol (g gland)$^{-1}$ (without

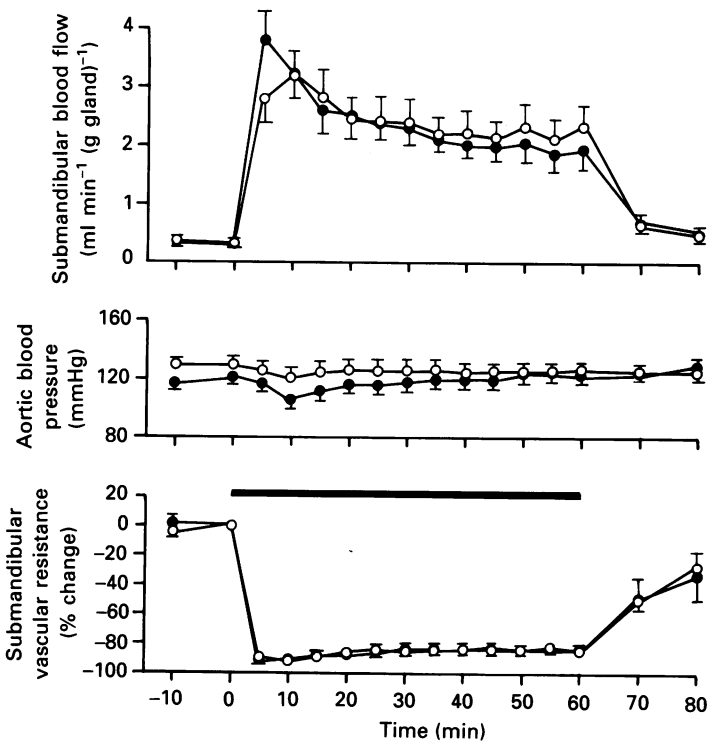

Fig. 2. Comparison of the changes in mean submandibular blood flow, aortic blood pressure and submandibular vascular resistance in response to stimulation of the peripheral end of the chorda-lingual nerve at 20 Hz for 60 min in anaesthetized ferrets. ○, normal control animals ($n = 10$). ●, animals given atropine (2·0 mg kg^{-1}; $n = 10$). Horizontal bar, duration of stimulation. Vertical bars, S.E. of each mean value.

atropine; $P < 0{\cdot}05$). In contrast, atropine completely abolished the increase in CGRP output in response to chorda-lingual stimulation (Fig. 3).

No increase in the outputs of VIP, substance P or CGRP from the submandibular gland were observed in response to stimulation of the chorda-lingual nerve at 20 Hz continuously in a group of five non-atropinized ferrets pre-treated with hexamethonium (20 mg kg^{-1}, I.V.). The secretory and vascular responses to chorda-lingual stimulation were almost completely abolished by the ganglion-blocking agent. Thus submandibular blood flow amounted to $0{\cdot}5 \pm 0{\cdot}1$ ml min^{-1} (g gland)$^{-1}$ both before and during stimulation. In three out of five of these animals a small initial secretion amounting to between just a quarter and half a drop of saliva was

produced during the first 2–3 min of stimulation and ceased thereafter. Peptide outputs were negative throughout. This large dose of hexamethonium did not block submandibular vascular or secretory responses to subsequent bolus I.V. injections of methacholine, or of the peptidergic agonists, administered via a femoral vein catheter, typical examples of which are illustrated in Fig. 4. Such bolus injections of VIP, substance P and CGRP produced a substantial fall in the arterial blood pressure

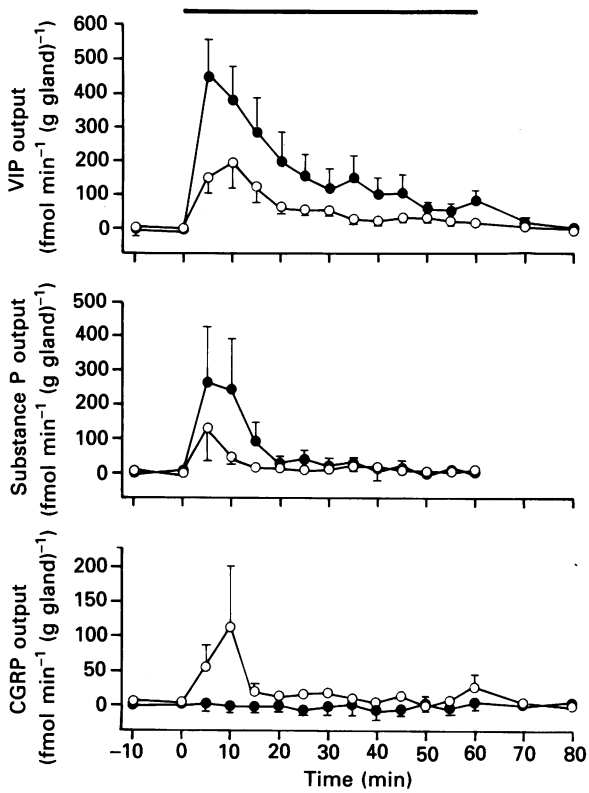

Fig. 3. Comparison of the changes in VIP, SP and CGRP output into the submandibular venous drainage in response to stimulation of the peripheral end of the chorda-lingual nerve at 20 Hz for 60 min in anaesthetized ferrets. ○, normal control animals ($n = 10$). ●, animals given atropine ($n = 10$). Horizontal bar, duration of stimulation. Vertical bars, S.E. of each mean value.

together with an increase in the flow of blood through the gland, showing that they exerted a more intense vasodilator effect on the submandibular vasculature than elsewhere in the systemic vasculature generally. Both substance P and CGRP also produced a secretory response under these conditions, but the gland was relatively unresponsive to CGRP and much higher doses were required to elicit this effect and the latency of the secretory response to the injection of CGRP was conspicuously longer (Fig. 4). In a group of four animals tested with bolus injections of CGRP the submandibular secretory response was found to be dose related; doses of 2, 5, 10 and

20 μg kg^{-1} eliciting flows of 0, 0·4 ± 0·4, 14·9 ± 8·1 and 18·9 ± 5·2 μl, respectively. The secretory effect of CGRP was not affected either by atropine or adrenoceptor blockade achieved, in this case, by pre-treatment with dihydroergotamine and propranolol (1·0 mg kg^{-1} of each).

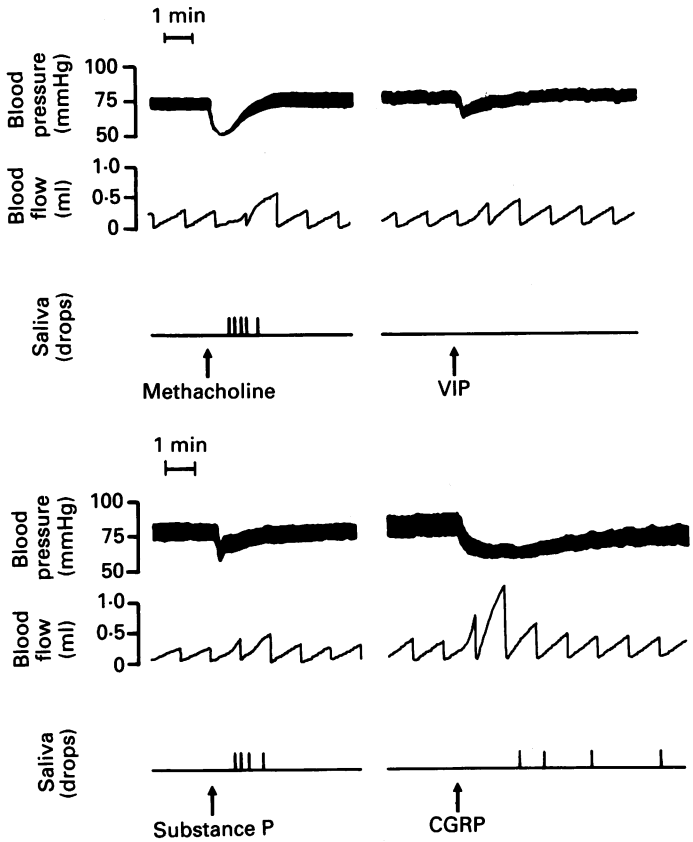

Fig. 4. Changes in aortic blood pressure, submandibular blood flow and salivary secretion in an anaesthetized ferret in response to I.V. bolus injections of methacholine (5 μg kg^{-1}; 26 nmol kg^{-1}), VIP (0·1 μg kg^{-1}; 30 pmol kg^{-1}), SP (0·1 μg kg^{-1}; 67 pmol kg^{-1}) and CGRP (20 μg kg^{-1}; 5·4 nmol kg^{-1}) following pre-treatment with hexamethonium (20 mg kg^{-1}).

Effects of a tachykinin antagonist

Intravenous injections of SP (0·1 μg kg^{-1}) elicited a flow of submandibular saliva amounting in total to 52 ± 11 μl in four atropinized ferrets, together with a fall in mean submandibular vascular resistance to a nadir of −48 ± 8%. Pre-treatment with the tachykinin antagonist (0·75 mg kg^{-1}, I.V.) completely abolished the submandibular secretory response to SP in atropinized ferrets (n = 4) and reduced the fall in vascular resistance from 48 to 18 ± 8%. This blockade was apparently quite specific because the tachykinin antagonist had no effect on the submandibular

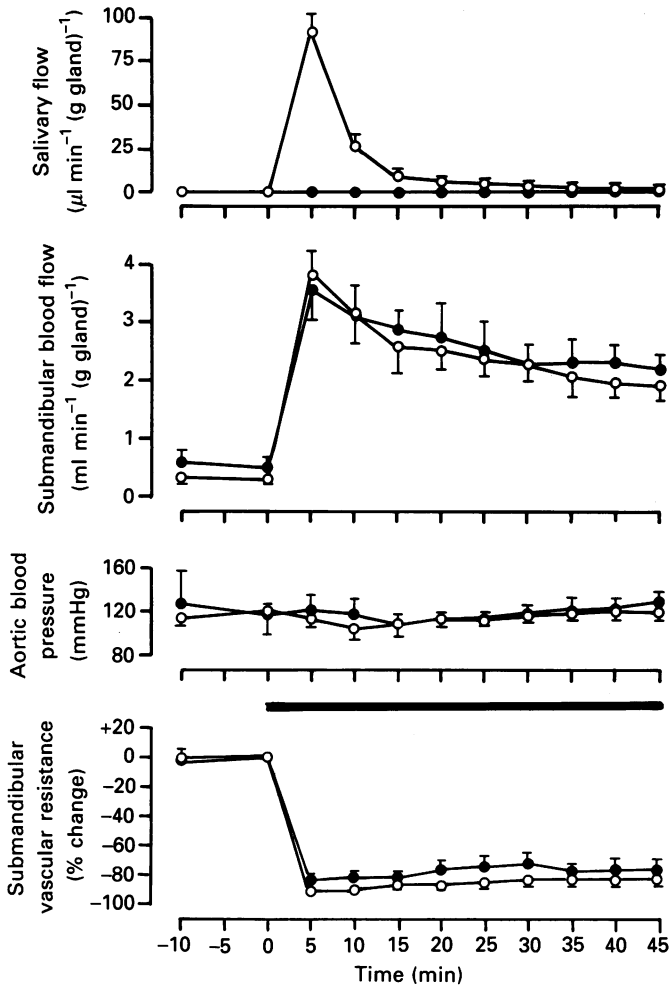

Fig. 5. Comparison of the changes in mean submandibular salivary and blood flow, aortic blood pressure and submandibular vascular resistance in response to stimulation of the peripheral end of the chorda-lingual nerve at 20 Hz for 45 min in anaesthetized, atropinized ferrets. ○, without a tachykinin antagonist (n = 10). ●, pre-treated with a tachykinin antagonist ([D-Arg^1, D-Cl^2 Ph^5, Asn^6, D-Trp^{7,9}, Nle^{11}]-SP; 0·75 mg kg^{-1}; n = 4). Horizontal bar, duration of stimulation. Vertical bars, S.E. of each mean value.

vasodilator response to I.V. VIP at a dose 0·1 μg kg^{-1} in atropinized ferrets (-21 ± 3% without the SP antagonist; 23 ± 9% with the antagonist). Under the same conditions, the tachykinin antagonist completely abolished the submandibular salivary secretory response to stimulation of the chorda-lingual nerve at 20 Hz for 45 min (Fig. 5*A*) without significantly affecting either the latency or the extent of the submandibular vasodilator response (Fig. 5*B–D*).

DISCUSSION

The results of these experiments confirm the fact that the submandibular gland of the ferret exhibits an atropine-resistant secretory response to stimulation of the parasympathetic innervation (Ekström *et al.* 1988*c*). However, the response is greatly reduced by comparison with that which is obtained in the absence of atropine, appearing only after a rather long latency (> 30 s) and fatiguing quite rapidly during prolonged continuous stimulation. The persistence of the vasodilator response, for as long as stimulation was continued in these animals, shows that this fatigue could not be due to failure of action potentials to invade the postganglionic parasympathetic nerve terminals. Furthermore, the fact that the secretory response to I.V. bolus injections of SP is not impaired, when that to nerve stimulation is exhausted (Ekström *et al.* 1988*c*) shows that the postsynaptic effector machinery is still capable of responding to an appropriate stimulus. Whereas the supply of the classical autonomic neurotransmitters, acetylcholine and noradrenaline, within nerve terminals can be replenished by synthesis locally or by reuptake, peptidergic agonists are thought to be synthesized exclusively in ribosomes in the nerve cell bodies and must then be transported to the terminals by axonal transport (see for instance Håkanson & Sundler, 1983). Such mechanisms are therefore more susceptible to fatigue when, as in the present study, nerves are stimulated following section and ligation. It was for this reason that the experiments in the presence of and in the absence of atropine were carried out in separate groups of animals, so avoiding the fatiguing effects of an initial period of stimulation prior to atropinization.

In the submandibular gland of the cat the atropine-resistant vasodilatation which occurs in response to stimulation of the chorda tympani has been attributed to the release of VIP (Bloom & Edwards, 1980; Lundberg *et al.* 1980). The fact that the output of VIP was also significantly raised in the present study and that the effect persisted throughout the period of stimulation, both in the presence and absence of atropine, strongly suggests that the submandibular atropine resistant vasodilator response is also due to the release of VIP in the ferret. The persistence of the response, which showed no sign of fatigue during continuous stimulation at 20 Hz for 60 min, is remarkable and suggests the presence of a large store of the peptide in the nerve terminals. The fact that nerve stimulation provoked a greater and more persistent increase in the output of VIP from the gland than of either SP or CGRP supports this contention. If, as we suppose, the vasodilator response is in fact due largely to release of VIP, far more of the peptide must be released initially than that required to produce a maximal increase in submandibular blood flow since the output of VIP steadily reduced during continuous stimulation at this high frequency, whereas the vascular response was well maintained. This is also suggested by the observation that the significantly greater output of VIP after atropine was not associated with any greater fall in submandibular vascular resistance.

The atropine-resistant secretory response, which was invariably well below that obtained in the absence of atropine, was completely abolished by pre-treatment with a tachykinin antagonist suggesting that it is mediated largely by SP, or a structurally related peptide. Further support for this contention is provided by the fact that the time course of the change in SP output in response to chorda-lingual

nerve stimulation in the atropinized ferrets was closely similar to that of the secretory response. However, it is also possible that release of VIP contributed to the volume secreted as the secretory response to I.V. injections of SP is greatly enhanced by concomitant administration of VIP in the ferret, even though VIP alone has no secretory effect (Ekström *et al.* 1988*c*). CGRP also evoked secretion of saliva in this species, when injected as a bolus alone, but only at a comparatively high dose, and it was found to be released within the gland in response to chorda-lingual stimulation in the absence of atropine. In the sheep CGRP has been shown to cause vasodilatation and to increase the concentration of protein in submandibular saliva (Edwards, Reid & Titchen, 1988). Furthermore, in the rat parotid gland CGRP releases amylase and potentiates secretory responses to both parasympathetic stimulation and SP (Ekström, Ekman, Håkanson, Sjögren & Sundler, 1988*a*). It is therefore possible that CGRP contributes to submandibular vascular and secretory responses when released together with other agonists from nerve terminals within the gland. The fact that the increase in the release of all these peptides from the submandibular gland of the ferret is completely abolished by pre-treatment with hexamethonium provides compelling evidence that they are all released from postganglionic parasympathetic nerve terminals and not from afferent nerve terminals. In this connection it is also noteworthy that pre-treatment with the sensory neurotoxin capsaicin in rats does not apparently affect the secretory response of the parotid gland to stimulation of the parasympathetic innervation (Ekström, Ekman, Håkanson, Luts, Sundler & Tobin, 1989*a*).

VIP is released into the venous drainage of the submandibular gland in response to stimulation of the parasympathetic innervation of the submandibular gland in the cat (Bloom & Edwards, 1980; Lundberg *et al.* 1980) and sheep (A. M. Reid, A. Schulkes & D. A. Titchen, unpublished observations reported by Titchen & Reid, 1990). Furthermore, in the ovine parotid gland, atropine resistant responses to reflex stimulation can be closely mimicked by the administration of exogenous VIP (Reid & Titchen, 1988), which suggests that the release of VIP exerts an important influence on salivary function under normal physiological conditions.

So far as we are aware the present study is the first in which SP and CGRP have been found to be released from any salivary gland in significant amounts in response to stimulation of the autonomic innervation. However, indirect evidence of such a phenomenon has been obtained for both SP and CGRP in the parotid gland of the rat, in which stimulation of the parasympathetic innervation continuously at 40 Hz reduced parotid SP content to about 60 and 25%, and CGRP content to about 75 and 65%, of the initial level after 20 and 60 min respectively (depletion of VIP follows a similar time course under these conditions and is of the same magnitude as that of substance P; Ekström, Brodin, Ekman, Håkanson, Månsson & Tobin, 1985). Furthermore, this relatively rapid depletion of peptide appeared to be correlated with fatigue of the non-adrenergic, non-cholinergic secretion which was obtained. More recently, it has also been correlated with loss of large dense-core vesicles from nerve terminals within the gland (Ekström, Garrett, Månsson, Rowley & Tobin, 1989*b*).

Pre-treatment with atropine was found to increase the output of both VIP and SP from the submandibular gland significantly in response to subsequent stimulation of

the parasympathetic innervation at 20 Hz continuously ($P < 0.05$). A similar effect was reported by Lundberg and his colleagues in relation to the release of VIP from the submandibular gland of the cat (Lundberg, Ånggård, Fahrenkrug, Lundgren, & Holmstedt, 1982) which they ascribed to a presynaptic muscarinic inhibitory mechanism. The present results suggest that a similar mechanism may also affect the release of SP. However, it clearly does not influence the release of all neuropeptides from autonomic nerve terminals because the output of CGRP from the submandibular gland was actually suppressed by atropine. It therefore seems that the output of CGRP from this gland during chorda-lingual stimulation is dependent, at least in part, upon an excitatory muscarinic mechanism.

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